

animals, while the same fluids unheated, are invariably and fatally infective in infinitely smaller quantities.

In Davaine's septichæmia in the rabbit, I have found throughout these experiments that the period of incubation is remarkably constant, death, after the first generation of infection by putrid blood, almost invariably occurring from about twenty to twenty-five hours, and consequently that if it does not occur within about that period, it may be concluded that infection has failed; it may die subsequently, as rabbits in confinement constantly do, more especially under the conditions in which they are kept in laboratories; but unless within about the period specified they do not die infected with specific septichæmia, the characteristic organisms are not found in the blood, nor is that blood infective; hence it is not necessary to observe such animals for more than a few days after inoculation.

These experiments were conducted in the laboratory at University College, with the co-operation of Dr. Burdon Sanderson, and at his suggestion.

XVII. "On the Development of the Enamel of the Teeth of Vertebrates." By EMILY NUNN. Communicated by Professor HUXLEY, F.R.S. Received June 14, 1882.

[PLATES 2-4.]

The question of the origin of the enamel of the teeth in vertebrate animals has been the subject of much discussion. It has been held—1st, that the enamel results from the calcification of the enamel cells (Tomes, Waldeyer, Edwards); 2nd, that it is formed by excretion from those cells (Kölliker, Hertwig, Leydig); and, 3rd, that it is not formed by these cells at all, but has the same origin as the dentine, whatever that may be.*

Again, there are numerous opinions concerning the nature and origin of the *cuticula dentis*:—1, that the cuticula is the persistent basement membrane itself (Huxley, Hertwig, Leydig); 2, that it is the altered enamel membrane; 3, that it is the external layer of cells of the enamel organ (Waldeyer); 4, that it is the metamorphosed *stratum intermedium* of the enamel organ (Löwve); 5, that it is the ends (*Deckeln*) of the cells of the enamel membrane (Köllman); 6, that it is a dermic structure (Tomes); 7, that it is an excretion of the cells of the enamel membrane (Kölliker).

* Huxley, "Tegumentary Organs," "Encyclopædia of Anat. and Phys.," vol. v, Sup., gives reasons for considering the whole tooth ecdemonic, but adds, "All these points can only be decided by a much more extensive series of investigations."

The existence of a basement membrane or *membrana præformativa*, said by some to cover the tooth papilla, is generally considered doubtful. It is asserted, 1st, that the newly-formed layer of enamel has been mistaken for it (Waldeyer); 2, that the first-formed layer of dentine has been mistaken for it (Löwve); 3, that the "appearances described are capable of a different interpretation" (Tomes).

The present investigation was undertaken at the suggestion of Professor Huxley with the hope of determining—or at least of getting more light upon—firstly, the history of the various membranes—the cuticula dentis, the so-called newly-formed layer of enamel, the *membrana præformativa*, and the first-formed layer of dentine; secondly, the origin of the enamel.

The present paper gives the results which have been arrived at concerning these points.

The nature of dentine will form the subject of a second paper.

Since it is well established that the teeth and placoids of the Plagiostomi are homologous with the teeth of the higher vertebrates, illustrations from these will be used as well as from mammalian teeth, adult and embryonic.

The drawings described have been made by the aid of the camera.

1. *The Cuticula Dentis*.—A membrane covering the enamel of young teeth has been repeatedly described as homogeneous and reticulated, or with "*zellzeichnungen*," supposed to have been made by adjoining cells. Huxley* says of this membrane: "It is perfectly clear and transparent, and under a high power exhibits innumerable little ridges upon its outer surface, which bound spaces sometimes oval, sometimes quadrangular, and about $\frac{1}{1500}$ of an inch in diameter. In the frog its surface is in parts reticulated as in man; in the mackerel and skate I have been unable to find any such reticulation. In the calf a similar membrane may be demonstrated, but it is much more delicate, and I have not seen the peculiar areolæ on its surface."

This account agrees with the description given by other writers of a membrane found lying upon the enamel, as also with my own observations.

The membrane, then, may be reticulated, entirely, in parts only, or not at all. It varies in thickness in the same animal, and as well as in different animals, and may be thick or delicate. It has been found by myself, as well as by other observers, upon the young tooth, both before and after its eruption. Hitherto it has not been figured,† neither has its history been determined; hence the various opinions as to its nature and origin. Waldeyer‡ declared it to be the newly-

* Huxley, "Quarterly Journal of Microscopical Science," 1853, pp. 157, 158.

† A drawing, by Nasmyth, has since been found, of the inner surface of this membrane, showing the impressions of the enamel prisms.

‡ Waldeyer, "Handbuch," 1869.

formed layer of enamel. Huxley* considered it as the persistent basement membrane.

Fig. 1, A, Pl. 2, is a drawing *en face* of this membrane as seen upon the molar of a young rabbit; it was taken from the tooth below the gum. The areolæ, upon its outer surface, were perfectly clear and transparent, as has been described, and a side view (fig. 1, B) showed them to be elevated into ridges. But the spaces or disks which they bounded were granular and were found to stain slightly with carmine. In this preparation they were round, but in some they are distinctly polygonal, while in others they are faintly seen and their outlines cannot be definitely traced. In the part of this membrane exposed above the gum, which appeared perfectly homogeneous, they could not be detected at all. While this membrane still covers the embryonic tooth "it may be that a few of the elongated cells of the organon adamantinæ adhere to it."† This may be the case even when the membrane has been well washed with a camel-hair brush previously to being hardened, as in fig. 1, B. This preparation clearly shows the reticulation to correspond in position to the cell-wall of the overlying cells and the enclosed spaces to the protoplasmic contents. The different structure of the clear colourless glassy areolæ and the granular slightly coloured disks, proves the reticulation to be something more than the impression of adjoining cells, as it has been described to be. The frequently granular character of the disks is rarely so distinct as in the present preparation, generally requiring a high power of the microscope for its demonstration, and it seems previously to have escaped observation.

The ridges, always upon the outer surface of the membrane, appear to be the walls of the cells, broken off a little above the extreme end of the cell, and in many preparations they occupy a larger relative space in the membrane than is shown in fig. 1.

It would seem as if the cell-wall were thickening about this end of the cell, or the protoplasm were undergoing a differentiation into a substance similar to cell-wall. The ends of the cells of the overlying enamel membrane frequently appear jagged (fig. 2), as also the surface of the membrane directed toward them, while its other surface, or that directed towards the enamel, is perfectly smooth, as is the case with the surface of the enamel, when the tooth has been sufficiently carefully and gradually decalcified.

In the upper part of fig. 2, where the tissues have not been separated, no membrane can be detected, the line between enamel cells and membrane not being a sharp one; but when the former are torn away, the latter is isolated (fig. 2, c), and a front view showed it to have the appearance of fig. 1, save that the disks are polygonal (fig. 2, B).

A similar structure on the tooth of the thornback is shown in fig. 3.

* Huxley, *l.c.*

† Huxley, *l.c.* p. 157.

Here, as is frequently the case, the ends of the cells appear broken or vacuolated, or striated, and with high power the striæ can be distinctly seen in transverse section on the edge of the membrane, which in all stages, being much more tenacious than the enamel cells, generally adheres to the enamel when the cells are removed, and may be raised up by the use of acid. As a rule, the older the tooth the more completely the reticulations or the cell-markings are obliterated, and the more resisting is the membrane, so that by maceration, as well as by the help of an acid, it may be taken off entire from the surface of the young tooth. It has been isolated by myself in this way, as also by Hertwig, who asserts it to be the "*derb*" basement membrane, and the "*zellzeichnungen*" upon it to be simply the impressions of adjoining cells. Preparations similar to fig. 3 clearly disprove this, and the basement membrane, when it can be demonstrated, is far from a "*derb*" structure, as will be shown further on.

Kölliker* says—"The ends of the enamel cells taken from the enamel present different appearances. Some are simply cut off squarely, others possess smaller (myself, Hertzig) or larger (Waldeyer) clear layers of the same breadth as the cells (the enamel fibres in process of formation), still others, finally, have pointed ends, with or without such layers (Tomes, Waldeyer, Hertzig). I consider these ends, which I also have seen, as artificial products—that is, as accidentally detached parts of the yet unfinished enamel fibres." If Kölliker had seen them on the firm dense membrane, as in fig. 3, or fig. 2, he could not have held this opinion. An extended and careful study leaves no room for doubt that this membrane, forming in many cases the cuticula of placoids and the cuticula dentis of both mammalia and fishes, is made by the metamorphosed ends of the enamel cells. But the cuticula has not always precisely this structure either upon the tooth or on the placoid; apparently more or less of the so-called enamel cells may enter into its formation. This, at least, is certain, the cuticula, or "*Schmelz oberhäutchen*," may not only have upon its surface the "cell markings," but it may be formed of entire cells (or at least of enough to include the nuclei) which have undergone a greater or less differentiation into horny tissue, obscuring more or less completely the outlines of the nuclei. Such a cuticula is shown *in situ* on the placoid represented in fig. 4. It is entire upon the under surface of the scute, which is more protected; upon the upper side fragments only are left. A high immersion lens showed distinctly on parts of the membrane, the outlines of cells and their nuclei (fig. 5) and the membrane could be traced into the columnar layer of epithelial cells at the base of the spine.

The cuticula of the mammalian tooth has several times been found

* Kölliker, "*Handbuch der Gewebelehre*," p. 385.

to have the same structure, and it has been possible, in transverse and longitudinal sections, to trace the gradual transition of the enamel cells into a perfectly homogeneous membrane (figs. 6 and 7, Plate 3), the cylindrical cells growing shorter as they approach the crown of the tooth, until, instead of being columnar, they are almost square, and finally flattened, and at last the outlines of the cells quite disappear, and there is left a perfectly homogeneous membrane.

These changes are not easy to follow; in many preparations it is impossible to make anything out, and the drawings have been made from most fortunate preparations selected from some thousands of sections prepared in various ways.

The cuticula dentis, then, is formed by the metamorphosis of more or less of the enamel cells, and this metamorphosis may begin before any calcification of the underlying dental tissues. In this stage it has been frequently taken for the "newly-formed layer of enamel," for the "basement membrane," and for the "first-formed layer of dentine."

2. *The Basement Membrane and Membrana Præformativa.*

The mucous membrane of the Plagiostomes immediately under the epithelium is frequently more or less laminated, and one of these laminae bounding the surface of the mucosa has often been described as the "*derb*" basement membrane. But being only the outer one of a series of laminae, it represents the basement membrane as simply the margin of the mucosa, with no definite and special structure of its own.

In order to get at the special structure of the surface of the derma supposed to be bounded by a basement membrane, it is well first to examine portions which have a cellular rather than a distinctly laminated structure, and it is absolutely necessary that the sections should be perfectly vertical to the surface of the derma, the study of which will be much facilitated if the epithelial cells have, for the most part, been previously washed away.

Fig. 8 is a vertical section of the dermis and of the base of a young tooth of the skate. The section being very thin and perfectly vertical, the structure of the cellular granular dermis stands out in strong contrast to the perfectly clear and dense basement membrane, brought into even greater relief by the cleft (*cl.*), and by its absence from a part of the derma where it has been accidentally torn away. The basement membrane runs up from the derma over the surface of the young tooth, which, as yet, has no calcific deposit, though there is a thick *dentinal basis* under the basement membrane, much thicker than the future enamel. It is finely granular throughout, showing no differences of structure in different parts, no signs of tubules, though

there is generally a faint granular striation running from the surface of the pulp to the basement membrane.

Fig. 9 is a portion of a vertical longitudinal section of the young tooth of a thornback hardened in alcohol. The basement membrane could be traced from the derma quite up to the apex on one side; it was quite invisible upon the other side, which was cut slightly obliquely to the surface of the tooth. The series of sections, however, furnishing the requisite conditions showed it to be there also. But in this section (fig. 9) not the slightest trace of it can be detected, and, but for subsequent sections, one could declare it did not exist.

If, at any time before calcific deposit, a young tooth* be carefully cut out, with a portion of the surrounding derma, and the cells of the enamel organ be washed away, examination with the microscope will show its outer surface to be quite smooth and dense,† and a clear strong homogeneous membrane, enveloping granular and cellular contents, can be pretty clearly made out. Pressure of the coverslip, causing the membrane to split, will bring out still more clearly the different structures of the perfectly clear granular transparent dense membrane, and the enclosed cellular pulp or granular dentinal basis upon its surface. If the tooth be now hardened in alcohol and cut into thin sections, those which are perfectly vertical will show the basement membrane of the small portion of adherent dermis to run up over the tooth as shown in fig. 8. It is exceedingly delicate and perfectly transparent, and when the surfaces of the tissues are cut at all obliquely, it is as invisible as a thin film of glass. Hence it can seldom be followed over the entire surface of even the most perfect section, and it cannot, like the cuticula in a somewhat older tooth, be separated by the use of acid. When a small bit is fortunately torn away with needles, it shows no "cell markings" upon it, but is perfectly smooth.

If a young tooth papilla be treated in this way, the basement membrane will be found quite upon the cellular pulp, a few cells of the enamel membrane frequently adhering to its surface. If the section be teased slightly and the membrane broken, the short outlines of its ends, sometimes standing out alone (Plate 4, fig. 10, *b. m.*), and its clear dense structure appears in marked contrast with the granular underlying pulp.

When the teeth, *in situ*, have been treated with chromic acid for a week or more, as is generally done, and then imbedded, and cut very much at random as is necessarily the case, it is a great chance if any trace of basement membrane can be seen. The enamel

* A small tooth of one of the Plagiostomes answers best—say of the thornback.

† At this stage the cuticula, if its formation be begun, is generally brushed off along with the enamel cells.

cells may appear to lie quite upon the cellular pulp; but if the section be good and sufficiently thin, a close examination will often reveal the membrane upon some part or other of the surface of the pulp.

Fig. 11 represents a section of a tooth papilla a little more advanced. A thin layer of dentinal basis (neither dentine nor enamel) covers the surface of the pulp, and over all is the basement membrane which, in one part, has fortunately been torn away with needles, and is shown to have a very different appearance from the cuticula, which later is developed above it, and can easily be isolated or "raised up" by acid.

In the Plagiostomes the soft dentinal basis under the basement membrane increases in thickness to a considerable depth before the beginning of calcific deposit, and thus affords a better opportunity for the study of the behaviour of the basement membrane, as well as for that of the formation of enamel and dentine, than is met with in mammalian teeth. It frequently becomes two or three times as thick as the future enamel will be before the occurrence of any calcification, which at last begins at the surface and proceeds inwards. The basement membrane of the derma can readily be traced running up over the surface of the young tooth at all stages, while the tissues are still soft. But its fate, after calcification begins, is more difficult to follow; whether it calcifies or remains uncalcified, cannot of course be determined from acid preparations, and from these alone it would seem that thin sections could be made. Experiment has proved, however, that alcoholic preparations of considerably calcified young teeth can, by sacrificing a razor for each section, and coming first upon the soft parts, then upon the hard, be cut into fine sections. These sections throw great light upon the manner of calcification and the relation of the soft and hard parts; they show, too, that after the removal of the cuticula no uncalcified membrane remains upon the surface of the tooth, but owing to the great refractive power of the calcified portion, it seemed impossible to demonstrate a thin membrane on its margin. The strong appearance of a membrane might still be simply the refractive lines. The persevering and careful use, however, of diluted nitric acid added under the coverslip, brings clearly into view the basement membrane upon the outer surface of the tooth; it is not "raised up," however, as is the case with the cuticula, if it has not been previously removed, and is an altogether different and much more delicate structure than the latter membrane. Fig. 12 is a section prepared in the way just described, showing the basement membrane, and at one place a fragment of the cuticula adhering to it. Teeth far enough developed to show a distinctly differentiated enamel layer with a broad band of dentine underneath, can, with sufficient skill, be cut into the thinnest sections; and, if these are vertical, the basement membrane may always be demonstrated on their surface or under the cuticula if it remain adherent.

Thus the basement membrane may be demonstrated running up from the surface of the dermis over the tooth papilla and young tooth in all its stages. The whole tooth is formed under the basement membrane and the entire growth is from the side of the pulp. The calcification begins at the surface of the tooth, and there is no addition made outside the part already calcified, no "newly formed layer of enamel" appearing as a membrane on the surface, the tooth receiving its additions from the side of the pulp only.

3. The Origin of the Enamel.

In its early stage the enamel cannot be distinguished from the dentine. It is formed by a later differentiation of a dentinal basis, which is the same for both dentine and enamel, and the nature of which can be best made out by teasing out, in salt solution, the young still soft tooth of some Plagiostome—say of a skate. It will then be found that the granular basis is formed by the regular arrangement side by side of the slender processes of the odontoblasts (fig. 13), and that they extend right up to the basement membrane, occupying the place of the future enamel as well as of that of the dentine; and it is these regularly arranged processes which cause the striæ often described. If the tooth be quite young, the processes are easily separated and seen in connexion with the cell from which they proceed, and they are frequently branched.

If the tooth be left in ammonium bichromate for a few hours, or even in salt solution, the processes, as well as the cells, can be more readily isolated. Indeed, on simply breaking through the basement membrane many of them escape and will be found outside.

When the tooth becomes a little older, and the *basis* thicker, the processes appear glued together by an intercellular matrix as it were, so that the whole basis appears as one mass, the constituent parts of which are separated with difficulty, and only by prolonged maceration in ammonium bichromate. But, by this time, the deposition of calcareous salts begins; and, soon after, the differentiation of the mineralised portion into dentine and enamel.

The fact that the processes of the odontoblasts extend quite up to the basement membrane, occupying the place of the future enamel, admits of only one view with regard to the origin of the enamel, and explains the existence of tubules which have often been described as extending into it, and continuous with the dentinal tubules.

The mode of calcification and the nature of dentine will be discussed in a future paper.

The results arrived at in this paper may be summed up as follows:—

1. The *cuticula dentis* is formed by the metamorphosis, either in whole or in part, of the enamel cells, which have nothing whatever to do directly with the formation of the enamel. In its early stages

the cuticula has frequently been considered as "the newly-formed layer of enamel" and also as the basement membrane.

2. The basement membrane may be demonstrated upon the surface of the tooth-papilla and upon the tooth in all stages of development. It becomes calcified with the other hard tissue of the tooth and cannot be separated by acid.

3. The enamel, like the dentine, owes its origin to the odontoblasts, the processes of which, in an early stage, may be traced quite up to its outer edge.

EXPLANATION OF FIGURES.

The same letters have been employed to mark corresponding structures in the whole series of figures.

The figures were drawn by the aid of the camera.

b. m. Basement membrane.

c. Cuticula dentis.

cal. Limit of calcification.

cl. Cleft formed by pressure of coverslip, dissecting needle, &c.

d. Dentine.

der. Derma.

di. Disks.

e. Enamel.

e. c. Enamel cells.

e. m. Enamel membrane.

ep. Epithelium.

e. l. External layer of the enamel organs.

e. o. Enamel organ.

f. Dentinal basis.

i. l. Intermediate layer of the enamel organ.

in. Involution of epithelium (enamel organ).

j. Jaw.

j. c. Cartilage of jaw.

n. Neck of enamel organ.

o. Odontoblasts.

p. Pulp.

pr. Processes of odontoblasts.

r. Reticulation.

ri. Ridges.

s. Space left by shrinkage of tissues.

t. p. Tooth papilla.

t. Tubule.

u. Limit of enamel layer.

Zeiss Oc. III was employed for all the sections. The objective is stated for the different figures.

Figure 1. Membrane lying between enamel and enamel cells of molar of rabbit.

A. *En face* view, the membrane is seen to be not homogeneous, but the reticulation and enclosed disks have a different structure.

B. Side view which shows the reticulation to be elevated into ridges. Two enamel cells and portions of others remain attached, fitting in between the ridges. Obj. $\frac{1}{16}$ immersion.

Fig. 5

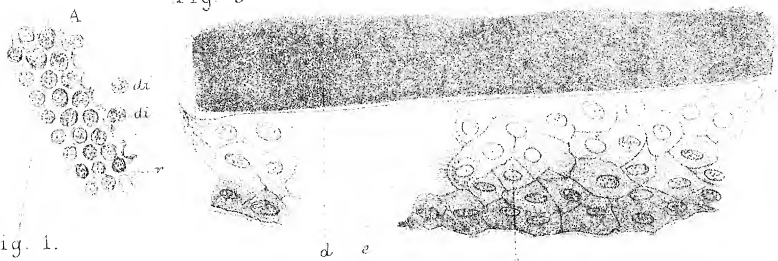


Fig. 1.

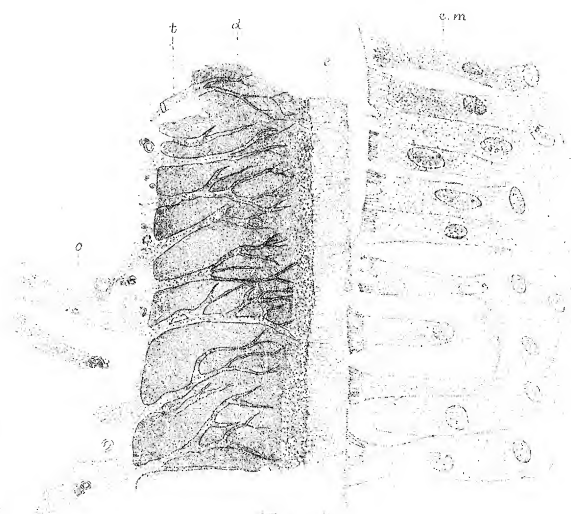


Fig. 3

Fig. 4.

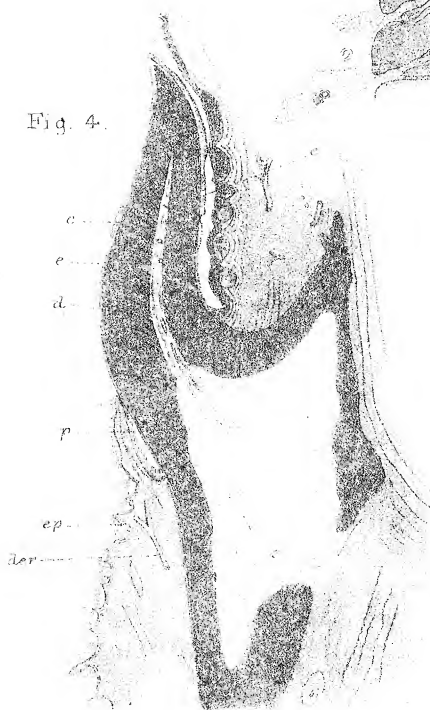
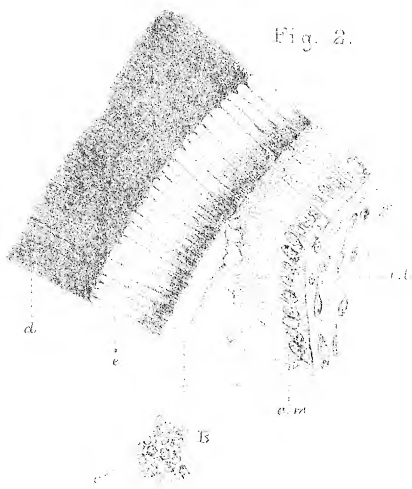


Fig. 2.



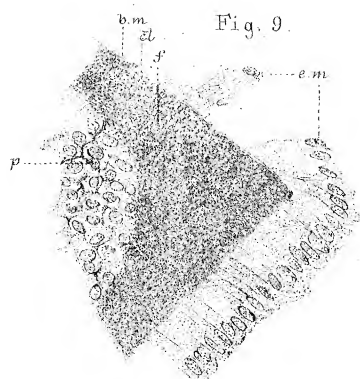


Fig. 9.

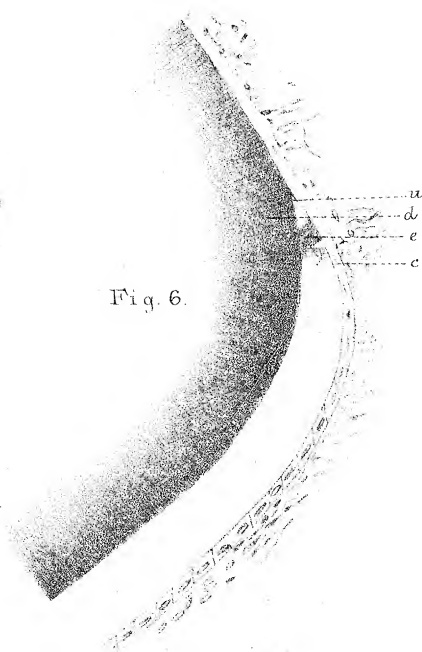


Fig. 6.

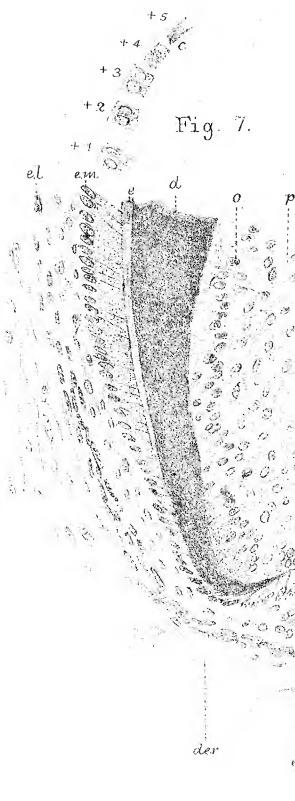
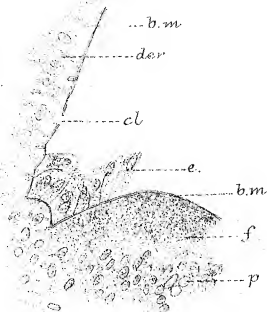


Fig. 7.

Fig. 8.



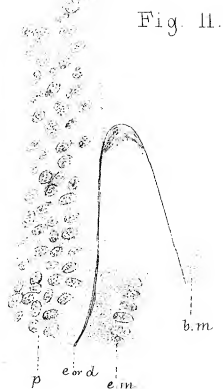


Fig. 12.

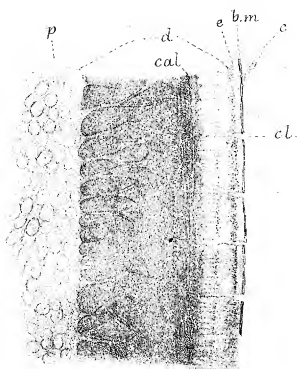


Fig. 13.

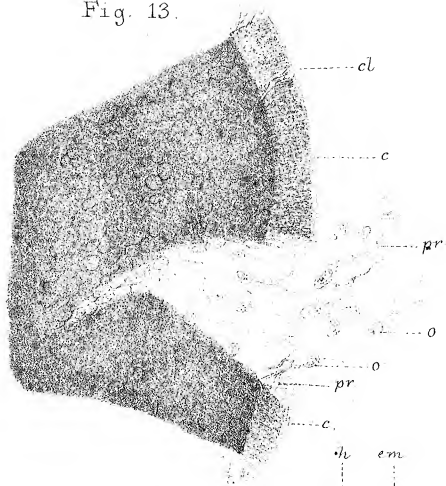


Fig. 10.

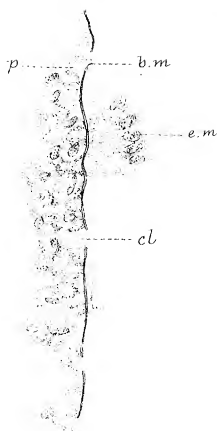


Fig. 14.

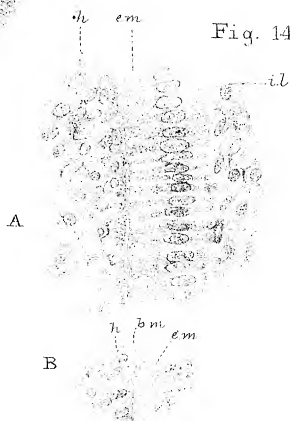


Figure 2. Part of transverse section of lower incisor of rabbit, showing the membrane (cuticula dentis) lying between enamel and enamel cells.

B. shows an *en face* view of a portion of *c*.

Obj. $\frac{1}{16}$ immersion.

Figure 3. A portion of vertical section of young tooth of an adult *Raia clavata* (thornback), showing the formation of the cuticula by the ends of the enamel cells. Obj. $\frac{1}{16}$ immersion.

Figure 4. Longitudinal section of spine from a fin ray of the thornback, showing the cuticula entire upon the under surface of the spine, with only fragments left upon its upper surface. Obj. 2 inch.

Figure 5. A portion of the cuticula of fig. 4, showing the outlines of the cells and their nuclei. Obj. $\frac{1}{16}$ immersion.

Figure 6. A portion of a transverse section of the lower incisor of rabbit, a short distance below the gum. The enamel extends over the side of the tooth no farther than *u*. A thick layer covered the front of the tooth which, except at one point, has disappeared. Obj. $\frac{1}{16}$ immersion.

Figure 7. A portion of a longitudinal vertical section of the upper small incisor of a rabbit. *e. m.*, 1, 2, 3, 4, 5, are cells of the enamel membrane drawn at intervals, showing their gradual change as they approach the crown of the tooth until, on its exposed portion, they form a homogeneous membrane. Obj. F. Zeiss.

(The following seven sections were from teeth preserved in alcohol, without acid.)

Figure 8. Vertical section of dermis and young tooth of *Raia batis* (skate). The basement membrane upon the derm stands out remarkably clearly, and could be seen running up over the young tooth quite to its apex. Obj. F. Zeiss.

Figure 9. The apex of vertical longitudinal section of tooth of a thornback, showing the basement membrane upon that edge which was cut exactly vertical to the surface of the tooth. No basement membrane can be distinguished upon the other edge, it being cut obliquely to the surface. Obj. F. Zeiss.

Figure 10. A portion of a vertical longitudinal section of a tooth papilla of a young skate. The cellular pulp still extends quite up to the basement membrane, which is more marked as the lines of the cells of the pulp appear to run at right angles to it. Obj. F. Zeiss.

Figure 11. Portion of a vertical section of a tooth papilla of a thornback, in which a thin layer of dentinal basis is seen under the basement membrane, a small piece of which has been fortunately torn away with needles. Obj. F. Zeiss.

Figure 12. Portion of section of a tooth of the skate. Calcification has proceeded from the surface as far as *cal*. The cracks in the calcified portion are produced in cutting. Most of the dentine is still uncalcified. Nitric acid was added under the coverslip and has brought out more clearly the basement membrane, which, however, never in any stage of the tooth can be raised up by acid, as can the cuticula which lies over it, a fragment of which adheres at *c*. Obj. F. Zeiss.

Figure 13. A portion of young tooth of a thornback, teased out in salt solution, showing the dentinal basis to be formed by processes of the odontoblasts arranged closely side by side. Obj. F. Zeiss.

Figure 14. Part of one of a series of vertical sections of tooth papilla of skate. In *B*, cut vertically, the basement membrane *b. m.* is distinctly visible. In *A*, cut more obliquely, it is not seen. Obj. F. Zeiss.

Fig 5



Fig 1



Fig 2



Fig 3



Fig 3

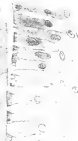


Fig 4



Fig 2



Fig 5

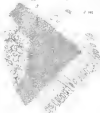


Fig 6



Fig 7



Fig 8





Fig. 12

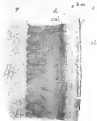


Fig. 13

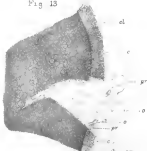


Fig. 10.



Fig. 14

